

Amendments to the Specification

Please amend paragraphs 0049, 0053 and 0054 as follows:

[0049] Examination of the sequence of L1R-M1R revealed a likely signal sequence at its N-terminus and hydropathy analysis suggested the existence of a membrane spanning domain beginning around position 187. Taking advantage of its natural signal sequence this protein was chosen as the N-terminal component of the polyprotein, thus insuring transport into the lumen of the endoplasmic reticulum and thereby exposing the polyprotein to glycosylating enzymes. Glycosylation can be important to native antigenicity of membrane proteins. This can also promote secretion into the culture medium, simplifying purification. Because neutralizing antibodies are expected to be directed against the extraviral segment of the protein, and to avoid the protein becoming anchored in the membrane, the amino acids after position 186 were not included in the construct. PCR was carried out using vaccinia strain WR as template. The 5' primer added ~~and~~ an AflII restriction site and the 3' primer changed the K at position 176 to the R found in the other sequences (see FIGS. 3 and 9) and added part of a GGGGSSGG (SEQ ID NO. 33) spacer-linker sequence following position 186, thereby incorporating a BamHI site near the 3' end of the amplicon. This product was then cloned into the plasmid expression vector PCDNA3.1©(+) (Invitrogen Corporation, Carlsbad, CA) between its AflII and BamHI sites. This plasmid was prepared by standard means and digested with AflII and EcoRI in anticipation of the three-fragment ligation described below.

[0053] To demonstrate cross-reactivity between vaccinia antibodies and LAA protein a competition ELISA was devised. ELISA plate wells were coated with vaccinia virus or elution fractions from a nickel column used to purify the LAA protein from culture supernatant (histag fractions). The wells were reacted with normal or vaccinia-immune sera from B6 mice in the presence or absence of a competitor. For histag fraction coated wells vaccinia virus was used as the competitor. For vaccinia virus coated wells histag fractions were ~~sued~~ used as the competitor. More specifically, the wells were

coated by overnight incubation with antigen in a buffer of 20 mM sodium borate, pH 9.5, at 2-8°C., the wells were washed twice with water and blocked with an ELISA diluent containing 2% normal goat serum and 5 mg/ml casein in tris-buffered saline. The wells were again washed twice with water and then incubated 3 hr. at room temp. with normal serum, immune serum, or immune serum plus competitor; final dilution of serum 1:100 in ELISA diluent. The plates were then processed as standard with biotinylated goat anti-mouse Ig, streptavidin-alkaline phosphatase, and PNPP substrate, and absorbance read at 405 nm. Competition was observed in both directions (see table below) indicating that anti-vaccinia antibodies can recognize the LAA protein.

[0054] Use of the Polyprotein as an immunogen. Groups of B6 mice are immunized IM with increasing doses (between 5 and 100 µg per dose) of the LAA polyprotein absorbed to aluminium hydroxide (Alhydrogel or BioVant™). Booster inoculations are administered repeatedly until high levels of virus-specific antibodies are obtained as judged by ELISA.